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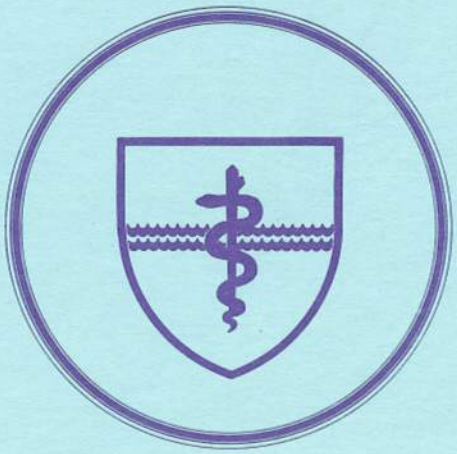

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CALCIUM and VITAMIN D METABOLISM IN SUBMARINERS:

Carbon Dioxide, Sunlight, and Absorption Considerations

by

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Naval Medical Research and Development Command
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CALCIUM AND VITAMIN D METABOLISM IN SUBMARINERS: CARBON DIOXIDE, SUNLIGHT, AND ABSORPTION CONSIDERATIONS

NSMRL Report No. 1037

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SUMMARY

In the prolonged submerged periods of early nuclear submarine patrols, significant derangements in calcium metabolism were noted, most notably a 50% decrease in urine calcium excretion that persisted for several weeks into the recovery period. A causal link was established between the elevated levels of ambient CO_2 and the behavior of calcium. An acid-base homeostatic mechanism was postulated but the decreased excretion could not be explained satisfactorily in terms of traditional acid-base theory and renal compensation of an acid-load. Later work, finding a readily exchangeable pool of CO_2 in bone in the form of bicarbonate ion, led to the proposal of a bone-buffering mechanism. During chronic low level hypercapnia, lower than the acid (CO_2) load required to trigger renal compensation, CO_2, with calcium following along, was deposited in bones via this buffering system. Cyclic deposition and release of calcium and CO_2 around the saturation point of this bone-buffering system was thought to explain the cyclic decrease/increase (but overall decrease) in urine calcium excretion. Investigations of possible ill effects from retained calcium have shown clinically insignificant calcifications of the kidneys, with other organs uninvolved.

More recent studies have implicated decreasing levels of 25(OH)-vitamin D as an alternative explanation for the decrease in calcium excretion seen later in patrol and in the post patrol periods. These studies have demonstrated a progressive decrease in 25(OH)vitamin D levels by 42% and a concomitant increase in calcium loss via the gut. This depletion of vitamin D stores was assumed to be the result of sunlight deprivation, since submariners are exposed to artificial fluorescent lighting with no ultraviolet component for 60-70 day periods. Yet no evaluation of the absorption of ingested vitamin D on patrol has been reported. A study which compares the vitamin D absorption patterns of 5 submariners on a normal diet at the beginning, middle, and end of a patrol is presented. It is concluded that absorption of vitamin D is not affected appreciably by the submarine environment, thereby providing stronger support for the proposal that the decrease in 25(OH) vitamin D levels is caused by lack of sunlight.

It is noted that $1,25(OH)_2$ vitamin D levels have not been measured in submarine studies and that the $1,25(OH)_2$ vitamin D precursors have only been studied separately without calcium measurement. As a result, the influence of vitamin D on the calcium behavior during submarine patrols has not been definitively established and requires more complete, simultaneous evaluation of all pertinent parameters. Paralleling the observation of the significant effect of no sunlight on vitamin D status are discussions of studies in which full spectrum lighting has shown a positive influence on physical and mental performance. Thus further research in the area may prove to be of benefit to known risk groups (e.g. elderly) and the submarine force in general. The submarine offers an ideal environment in which such research can be continued.

ABSTRACT

A 42% decrease in 25(OH)vitamin D levels has been noted in subjects over the course of submarine patrols and is thought to be the result of prolonged sunlight deprivation. The influence of the submarine environment on absorption of ingested vitamin D has not been previously investigated. This study examines the vitamin D absorption patterns of 5 subjects at the beginning, middle, and end of a 69 day patrol on a normal diet and with no exposure to sunlight. It was found that the magnitude and pattern of absorption does not change appreciably. It is concluded that the absorption of vitamin D is not affected by the submarine environment and that any drop in 25(OH) vitamin D levels seen during patrol is caused by lack of sunlight, ultraviolet light in particular. Implications in terms of sunlight deprivation and calcium metabolism are discussed in a historical review of calcium and vitamin D investigations on submarines.

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ADMINISTRATIVE INFORMATION

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The introduction of the nuclear-powered submarine in the 1950's significantly enhanced the capabilities of naval forces. The ability to remain submerged for months at a time provided a valuable operational advantage. Prolonged submerged periods, however, also exposed crewmembers to numerous physiological stressors including radiation, lack of sunlight, increased CO_2 levels, atmosphere contaminates, and loss of external time cues.[8] Concern for the potential ill effects on the health of these individuals prompted numerous investigations which concluded that the overall health of submariners was not compromised and that basic hematologic and biochemical parameters remained in the normal ranges.[44] Nonetheless, statistically, if not clinically, significant relationships were demonstrated and, because of their potential to elucidate more subtle biochemical interrelationships, several metabolic parameters were studied in depth.

One such parameter is calcium metabolism. Early work indicated significant derangement of calcium metabolism in the submarine environment and postulated several mechanisms including acid-base/bone buffering, parathyroid hormone, diet, and activity levels. More recent advances in clarifying the action of vitamin D led to suspicions that vitamin D had a significant role in the altered calcium metabolism as well.

This paper proposes to investigate further the metabolism of calcium in submariners, in particular, the role of vitamin D. First, a brief historical discussion of the calcium/vitamin D problem will present pertinent background information. Second, a study conducted during a recent Trident submarine deterrent patrol, involving vitamin D levels and Ca absorption, will be introduced. Finally, the results of the study will be examined and evaluated in relation to previous investigations.

BACKGROUND - CALCIUM

The submarine environment is a closed system and depends on air revitalization equipment to maintain the atmosphere. This equipment can only approximate the normal open atmosphere and, until recently, CO_2 levels of 0.5 - 1.5%, well above the normal 0.03%, were common. As early as 1963, these elevated CO_2 levels were found to significantly affect calcium metabolism. The "Operation Hideout" experiment exposed humans to 1.5% CO_2 for six weeks and found that urine calcium excretion decreased by 50% and that this decrease continued for four weeks into the recovery period.[39] These surprising results directed further research into three different paths. First, any proposed mechanism for the decreased excretion would have to explain the continued decrease four weeks after exposure. Second, in view of normal calcium intake[10], the mechanism must also explain the disposition of the "retained" calcium in the body. Third, potential health hazards from the retained calcium in the form of tissue or organ calcifications would require assessment.

Three separate studies, exposing subjects to CO_2 concentrations from 0.7% to 1%, disclosed an apparent cyclic variation in calcium homeostasis during chronic low level hypercapnia.[19,20,31] Summarized in the following graph, these studies showed that both serum calcium levels and urine calcium excre-

tion followed approximate 20 day cycles with an overall slight increase in calcium levels and substantial decrease in calcium excretion.

TABLE 1
Calcium parameters in low level hypercapnia
(summarized from reference 19, 20, 31)

DAY	0.20	20-40	40-60	Overall
plasma calcium	↓	↑	↓	↑
urine calcium excretion	↓	↑	↓	↓

In addition, it was noted[20] that urinary acid secretion roughly paralleled urinary calcium excretion.

Gray attempted to explain the data by postulating a renal mechanism.[20] Chronic exposure to low levels of CO_2 presented a mild CO_2 (acid) load to the kidney, apparently lower than that necessary to trigger the appropriate response of increased acid excretion, leading to mild H^+ retention. Since calcium excretion paralleled H^+ excretion, a similar renal response of calcium retention via "enhanced tubular reabsorption" was assumed. After about 20 days, a threshold of mild acidosis was reached and led to an appropriate response of increased H^+ excretion coupled with increased Ca^{++} excretion -both mild. After 40 days, enough excretion had taken place to lower the acidosis below the threshold resulting in decreased Ca^{++} and H^+ excretion.

An inefficient "hunting mechanism" in the kidney thus explained the apparent cyclic response to chronic low level hypercapnia. As the stimulus increased in intensity, i.e. increased CO_2 concentration, this hunting mechanism would get more efficient in its response. This prediction was borne out in studies at higher CO_2 levels in which, at 3% CO_2,[37] calcium excretion increased at day 4-5 instead of day 20 and, at 4% CO_2,[18] calcium excretion actually remained elevated throughout the study.

Gray's proposal, however, could not satisfactorily explain the continued suppression of calcium excretion into the recovery period, although he did suggest a possible bone CO_2 buffering system. Nevertheless, his study strengthened the idea that CO_2 and calcium were related via an acid-base homeostatic mechanism and indicated that the usual, expected renal response to acute acid-base challenges did not always apply in chronic, low level exposure situations.

It had long been known that bone was a large reservoir of both CO_2 and calcium. As early as 1964, investigators postulated that this dynamic reservoir played a role in the cyclic CO_2/calcium interrelationship seen in low level hypercapnia[7,39,40] and in the persistent renal response in the post-exposure period.[20] Poyart[33,34] studied the bone CO_2 content in rats and found that bone CO_2 existed in two different forms: relatively stable, slowly exchangeable carbonate ($CO_3^=$) in the crystals (60-70%) and bicarbonate (HCO_3^-) in the hydration shell of the hydroxyapatite crystals

(30–40%). The bicarbonate pool was shown to be rapidly exchangeable by $^{14}CO_2$ experiments. The equation for this exchange is depicted below.

$$CO_2 + H_2O + CO_3^{=} \rightleftharpoons H^+ + HCO_3^- + HCO_3^-$$

$$CO_2 + H_2O + CO_3^{=} \rightleftharpoons 2HCO_3^-$$

The rate-limiting step in the exchange appeared to be the relatively low blood flow in bone. The resulting slow exchange would prevent the large CO_2 bone stores from playing a significant role in buffering the extracellular fluid in substantial, acute acid-base abnormalities, but would not prevent the participation of this buffer system in chronic low level CO_2 exposures.

Using the above finding, Schaefer attempted to explain the calcium/CO_2 relationship solely on the basis of bone buffering.[31,36] Initially, in low level CO_2 exposure, the acid load was small, not enough to trigger the threshold of usual renal regulation. The CO_2 load was therefore handled by bone absorption via the buffer equation depicted previously. After about 3 or 4 weeks, this buffering system became saturated. CO_2 was then released from bone and raised the acid load above the renal threshold for compensation, thus explaining the slight increase in H^+ excretion during days 20–40. By day 40, enough excretion occurred to decrease acid load below the renal threshold and CO_2 uptake in bone increased, again decreasing acid secretion. Schaefer additionally proposed that calcium followed this cyclic handling of CO_2: absorbed in bone during the bone buffering stages (and thus poorly excreted) and released from bone and excreted by the kidneys during the renal compensation phases. The proposal gained strength with a finding in a guinea pig study during exposure to 0.5% CO_2 that showed an increase in plasma calcium along with a decrease in bone calcium content corresponding to the bone release/renal compensation phase.[38] Thus, the bone phenomenon was primarily an acid-base regulatory mechanism, with calcium following along passively.

In the course of his investigations, Schaefer also found that the mobilization of calcium from bone after the CO_2 bone buffering system was saturated coincided with an increase in kidney calcium content and renal calcifications in the guinea pigs.[38] Studies investigating the possibility of calcium deposition in other tissues, however, showed that the lungs, heart, and liver were not affected and the focal and tubular calcifications noted in the kidneys produced no significant tissue damage and were of no clinical significance.[41]

Schaefer's work, outlined below, offered a workable mechanism for the apparent cyclic response of calcium metabolism to CO_2 and provided insight into its clinical implications, in particular, the concern about damaging tissue calcifications. Yet his proposed mechanism did not adequately explain the continued suppression of calcium excretion for several weeks after exposure ends. Once the CO_2 stimulus has ended, the need for the bone buffering system ceases and CO_2, along with calcium, should be released from bone and excreted. This excretion does not occur for 4–5 weeks, however, and Schaefer explained that this phenomenon was due to the slow turnover of CO_2 and calcium stores in bone. He readily admitted that such a cyclic, slow-

turnover buffering mechanism was not consistent with traditional acid-base concepts but explained that the behavior was probably due to the extremely low CO_2 levels involved.[36]

BACKGROUND - VITAMIN D

Schaefer's theory, however conjectural, does explain most of the findings in submarine investigations. Nevertheless, the assumption that this mechanism is the only one operative in the complex submarine environment is not warranted. Other potential contributing factors have been identified and investigated. The three hormones that play a role in calcium homeostasis, parathyroid hormone, calcitonin, and vitamin D, could only recently be investigated because of prior limitations in performing accurate assays. Messier has shown that calcitonin and PTH levels do not vary from normal during a submarine patrol, but the assays were only accurate to 15%.[31] Schaefer, though, has postulated that PTH, with more minute variations, is the causative factor in the calcium deposition in the kidneys, since tissues without PTH receptors, e.g. heart and liver, are not affected.[41] Obviously, more refined assays are required in order to identify any finer variations.

Vitamin D assays, on the other hand, have become precise enough to demonstrate the significant variations in vitamin D levels on submarine patrols suspected as early as 1974. Davies proposed that, since vitamin D metabolism is dependent on ultraviolet light and, for 60-80 day periods, submariners are exposed to artificial light, with no ultraviolet component, vitamin D levels would be affected and so have an effect on calcium homeostasis.[9] Indeed, Preece, in 1975, found that 25(OH)vitamin D levels in eight submariners dropped from 13.7 ng/ml to 7.9 ng/ml over 60 days (mean values).[35] Davies, using nine men in a chamber for 10 weeks, also found a similar drop in 25(OH)vitamin D levels.[10] He further noted that, from week 5 on, fecal excretion of calcium progressively increased, paralleling the decrease in 25(OH)vitamin D levels.[11]

The findings above now raise the question whether the overall effect of the submarine environment on calcium metabolism is one of calcium retention, via the decreased kidney excretion mechanism proposed by Schaefer and others, or an overall calcium depletive process, caused by a decrease in vitamin D and therefore an increase in calcium loss via the intestines. The calcium loss would then explain the renal retentive response of decreased excretion. Unfortunately, the difficulties in obtaining precise determinations of mineral balance only compound the problem. In addition, although such a vitamin D mechanism could explain a continued decreased renal calcium excretion into the recovery period (until vitamin D levels have been restored), the renal response of decreased calcium excretion occurs much too early, 2-11 days in Gray's studies of 1969 and 1973[19,20], to be explained by vitamin D deficiency. A decrease in vitamin D levels sufficient to have an effect on calcium levels would take much longer than 2 days to develop. Davies has even suggested that both of the above mechanisms are operative in the submarine environment, the CO2/acid-base mechanism initially, then the vitamin D depletion mechanism later, explaining the "cyclic" response of decreased renal calcium excretion occurring around week 7 and the continued excretion deficit into the recovery period.[10]

In order to put the above findings and proposals in a clearer perspective, a brief presentation of the role of vitamin D in calcium metabolism is in order. Over the past two decades investigators have clarified the action of vitamin D and traced its complex metabolic pathway. The basic pathway and interrelationships are depicted in the diagram on the following page. Perhaps the most striking conclusion from these studies is that vitamin D functions not as a vitamin, a cofactor in enzymatic processes, but as a hormone. It is manufactured in one organ, transported by the bloodstream to have a physiologic effect on some other part of the body, and regulated in its formation by the feedback from the product of its action, serum calcium.[12]

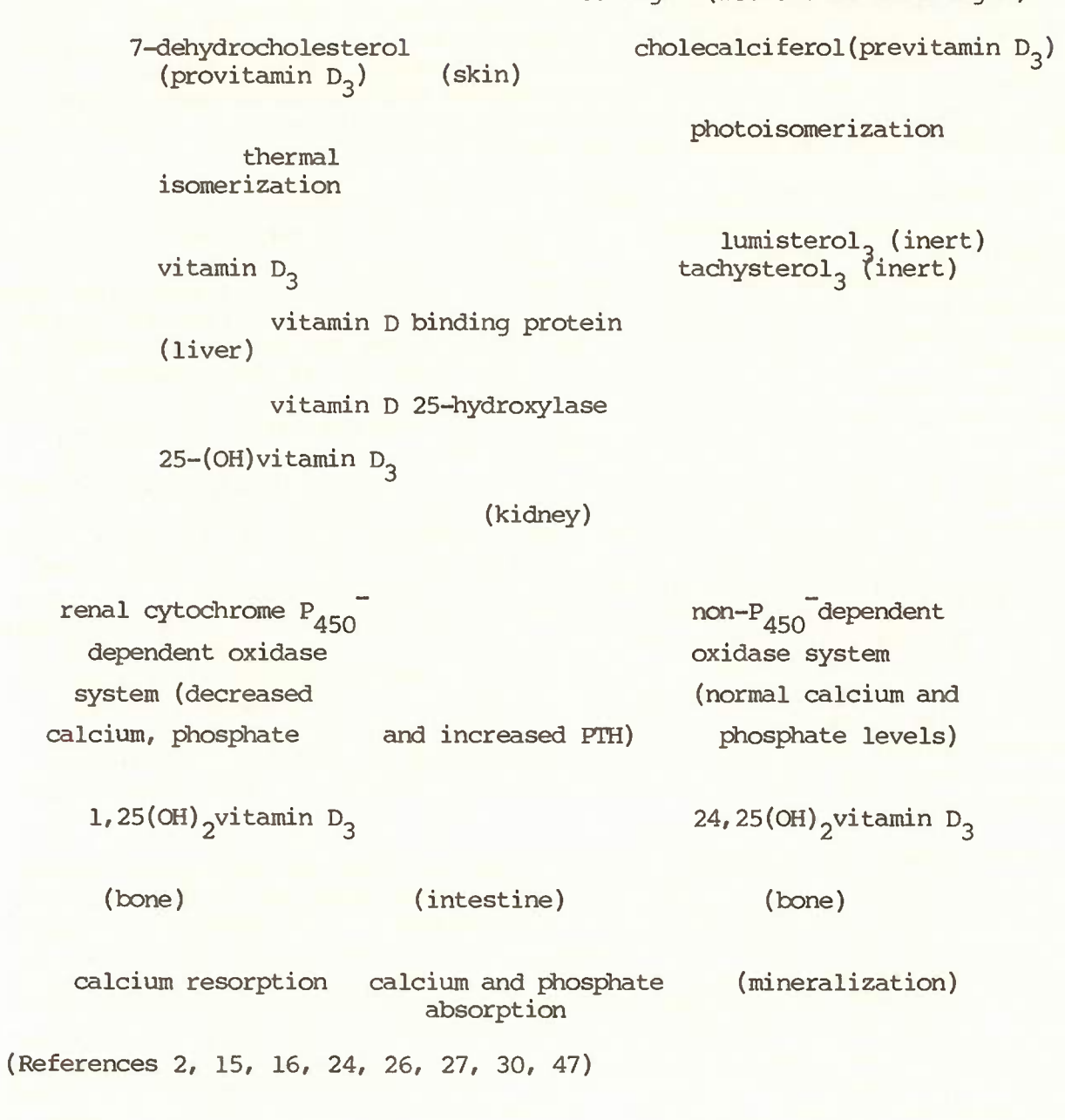
As shown in the diagram, 7-dehydrocholesterol in the skin is converted by ultraviolet light to cholecalciferol, which in turn is converted by light to biologically inert metabolites and by body heat gradually to vitamin D_3. This branching photoisomerization pathway is purported to be a mechanism which prevents buildup of toxic amounts of active vitamin D metabolites during prolonged ultraviolet light exposure[27] and which allows for sustained production of vitamin D after a brief exposure.[26] The vitamin D_3 is then transported to the liver where it is hydroxylated to 25(OH)vitamin D_3, which has a very low biological activity but is transported to the kidneys where the pathway diverges again. If the calcium/phosphate status of the body is normal, hydroxylation will take place at the 24 site and result in $24,25(OH)_2$vitamin D_3. This compound likewise has minimal if any biological effect, although Ushakov, et al has recently demonstrated a potential role of this metabolite in bone calcium deposition, i.e. mineralization.[46] If, on the other hand, calcium or phosphate levels are below normal, hydroxylation will take place, via a different enzymatic system, at the 1 site, resulting in $1,25(OH)_2$vitamin D_3, the metabolically active form of vitamin D. The parameter which determines the direction of the pathway at the second hydroxylation step is actually the phosphate level more than calcium. Low phosphate levels stimulate the production in favor of $1,25(OH)_2$vitamin D_3, whereas high phosphate levels stimulate the $24,25(OH)_2$vitamin D_3 pathway. Parathyroid hormone exerts its effect by determining the phosphate level intracellularly in the kidney[12,26]

Once formed, the $1,25(OH)_2$vitamin D_3 has an effect on two target organs, bone and intestine. In the intestine, the hormone acts via an RNA-induction mechanism similar to that of other steroid hormones.[47] A membrane receptor complexes with the hormone and transports it to the nucleus where RNA-induction occurs to increase the production of the calcium binding protein.[3] The overall effect is increased calcium and phosphate absorption from the gut.[24] In the bone, $1,25(OH)_2$vitamin D_3 acts to increase calcium resorption from already mineralized bone in order to make it available for mineralization elsewhere and thus allow bone remodeling. A role in calcium deposition during remodeling has not been conclusively demonstrated nor has any direct effect of $1,25(OH)_2$-vitamin D_3 on the kidney.

In addition to the skin production of vitamin D outlined above, oral intake of the hormone plays a role. Oral vitamin D takes two forms, D_3 and D_2. Natural vitamin D_3 is found in fish and certain grains but only in very small amounts. Vitamin D_2, synthesized by ultraviolet irradiation of a fungus sterol, ergosterol, has been used, particularly in the United States, to

DIAGRAM

VITAMIN D PATHWAY



(References 2, 15, 16, 24, 26, 27, 30, 47)

fortify various foods. This compound undergoes the same 25-hydroxylation as vitamin D_3[43] and the resulting 25(OH)vitamin D_2 cannot be distinguished from 25(OH)vitamin D_3 in normal assays[23] nor in observed biologic activity.[35] Neither of these compounds, nor both together, when taken orally, have a dominant role in overall vitamin D status. Several investigators have shown that sunlight has a greater effect on 25(OH)vitamin D levels than diet.[17,43] Even in high levels of vitamin D_2 supplementation, endogenous production is the principal source of 25(OH)vitamin D in healthy adults.[23]

The pathways and characteristics of vitamin D metabolism explained above clarify the results and discussions of the submarine vitamin D studies presented previously. The results of these studies suggest that, during a two to three month patrol period involving complete isolation from natural light, 25(OH)vitamin D levels decrease to the point where intestinal absorption of calcium is affected. As a result of the calcium loss via decreased absorption from the gut, the kidneys respond by decreasing calcium excretion. The decreased renal calcium excretion observed late in patrol, previously explained by Schaefer and others as a cyclic response of a bone buffering system to a decreased (i.e. subthreshold) CO_2 load on the kidneys, may thus be explained by depletion of total body calcium caused by decreased vitamin D levels. Moreover, the mechanism explains the continued decreased renal calcium excretion into the recovery period, since a delay would be involved in replenishing vitamin D levels and so calcium stores.

Davies' vitamin D mechanism very readily complements the bone buffering/acid-base mechanism in explaining the effects of the submarine environment on calcium metabolism. Yet, further study is necessary in order to define more clearly the calcium/vitamin D relationship on prolonged submarine patrols. The following investigation, conducted on board a United States Trident-class submarine during a recent deterrent patrol, examines one area of concern: the role of sunlight versus diet in vitamin D status. While it is well demonstrated that sunlight-induced endogenous vitamin D production is the more important source of the hormone, it cannot be assumed that the decrease in vitamin D levels seen during patrol is caused entirely by lack of sunlight. Does the submarine environment somehow affect the handling of ingested vitamin D as well? The study seeks to establish whether vitamin D absorption changes over the course of a 70 day patrol in subjects on a normal American (vitamin D fortified) diet and so provide an indirect indication of the magnitude of the effect from lack of sunlight.

METHODS

The subjects of this study were 10 caucasian male volunteers from the crew of the USS OHIO (SSBN 726) GOLD during a 69 day patrol. The volunteers agreed to participate after appropriate briefings and written informed consent was obtained in accordance with the policies of the Human Use Committee of the Naval Submarine Medical Research Laboratory (NSMRL), Groton, CT. Each subject was tested during three testing periods which began on days 3, 31, and 59, respectively. In preparation for each testing period the subjects were instructed to abstain from all dairy products for a minimum of 48 hours and to abstain from all foods, caffeine, and cigarettes for the 12 hours immediately preceding the initial blood sampling. Each participant kept a dietary intake

log in order to aid in and document compliance with these dietary restrictions. At the time of the initial sampling (our "control" or baseline value) each subject was given 50,000 I.U. of vitamin D_2 orally. At 6, 12, 18, 24, and 48 hours after the ingestion, blood was again drawn from each subject. The specimens were allowed to clot, then centrifuged, and the serum was collected into individual vials and immediately frozen. The specimens were kept frozen until the end of patrol and, upon arrival in port, transferred in the frozen state to the Endocrine Unit, Vitamin D Laboratory, Massachusettes General Hospital, Boston, MA where vitamin D analyses were conducted by Dr. Michael Holick.

RESULTS

The original intent of the study presented in the METHODS section involved 10 subjects, which would probably have allowed quantitative statistical analysis of the baseline and post-ingestion vitamin D levels. Unfortunately, due to unforeseen difficulties in processing, many of the specimens were rendered unusable for valid analysis. Since the vials were randomly numbered, many of the absorption studies are incomplete. As a result only the studies which are relatively complete are depicted in Table II on the following page. Because of the low number of subjects, statistical evaluation is not possible. However, the reasonably complete studies available appear to be consistent and offer the opportunity for qualitative assessment and discussion.

The numbers in the table represent total vitamin D levels (vitamin D_2 + vitamin D_3) in nanograms/milliliter with an intra-study accuracy of 10% and an inter-study accuracy of 13%. Zero (0) values represent no detectable vitamin D (less than 2 ng/ml). Subject III inadvertently did not take the vitamin D capsule during the second testing period, an event which was actually fortuitous in that his values provide a qualitative control for the study. In each of the subjects studied it was observed that vitamin D levels rose to a peak over the initial 12-18 hours after ingestion then began to fall over the rest of the 48 hour testing period. This is graphically depicted in Figure 1. During the "control" period, on the other hand, no vitamin D was detected on any of the analyses indicating that the values obtained in the other studies were the result of the ingested vitamin D_2. Moreover, it was noted that, over the course of the patrol, the absorption patterns and magnitude did not change appreciably, suggesting that the absorption of vitamin D_2 from the gut was not affected by the submarine environment. It is also interesting to note that in four of the subjects the baseline (pre-ingestion) levels were 0 in all three testing periods and varied from 0-10 ng/ml in Subject 1. These low initial values significantly reduce the possibility of interference from previous, undisclosed vitamin D ingestion or ultraviolet light exposure, since vitamin D levels reflect recent significant exposure via oral ingestion or ultraviolet light.[1]

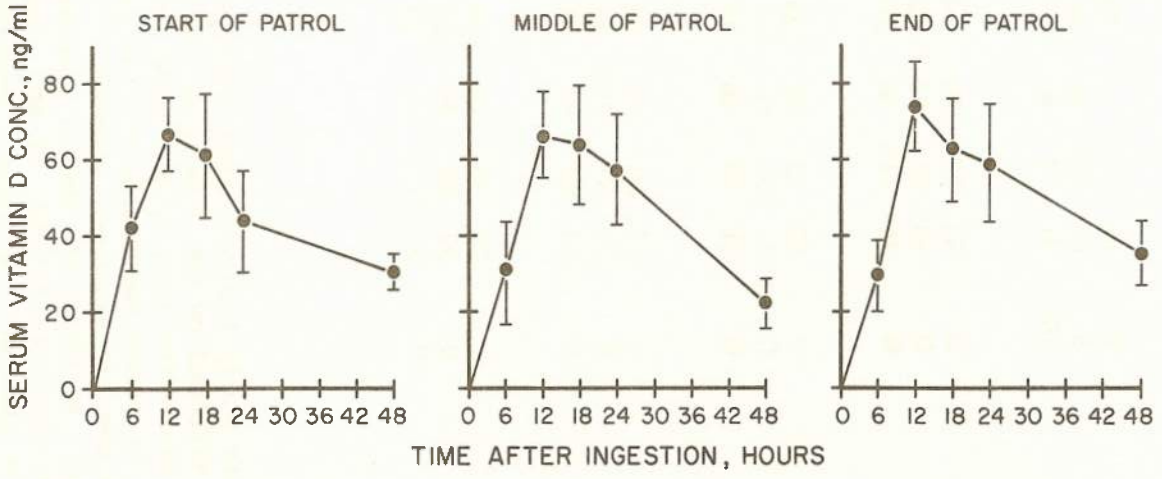


FIGURE 1. Vitamin D levels measured over 48 hours after ingesting 50,000 I.U. on the 3rd, 31st, and 59th day of a 69-day submarine patrol. Before ingestion, control measurements showed a zero concentration.

TABLE II

Vitamin D levels in ng/ml
after ingestion of 50,000 I.U. of
vitamin D_2 at hour 0

HOUR	00	06	12	18	24	48
SUBJECT I						
DAY 3	0	68	73		57	22
DAY 31	5	0	56	64	59	19
DAY 59	10	21	77	41	37	23
SUBJECT II						
DAY 3	0	63	77	80	54	32
DAY 31	0	54	84	66	52	26
DAY 59	0	45	110	98	103	46
SUBJECT III						
DAY 3	0	42	72	54	23	--
DAY 31	0	0	0	0	--	--
DAT 59	0	51	79	56	50	60
SUBJECT IV						
DAY 3	0	14	83	93	69	38
DAY 31	0	29	87	102	95	39
DAY 59	0	33	69	91	84	39
SUBJECT V						
DAY 3	0	25	30	19	16	--
DAY 31	0	39	40	25	24	8
DAY 59	0	0	37	30	20	14

DISCUSSION

The findings of this study indicate that absorption of injested vitamin D from the gut is not affected by the submarine environment over the course of a 70 day patrol. Since the only other known source of vitamin D is endogenous production, it seems reasonable to conclude that the decrease in 25(OH)vitamin D levels seen in previous studies is caused by a defect in that production pathway. Although the defect can conceivably be anywhere in the production process, there is little, if any, reason to suspect significant alteration in the enzymatic systems themselves. It is much more plausible to attribute the decrease in production to a lack of ultraviolet light, which is not a component of the fluorescent lighting used on submarines but which stimulates the initial reaction of the entire pathway. This study then, qualitatively at least, resolves one of the basic questions of vitamin D behavior on submarines, namely the cause of the progressive decrease in levels. In addition, the conclusion appears to be consistent

with Davies' proposal that a decrease in calcium excretion late in patrol and during initial recovery is due to a decrease in 25(OH)vitamin D caused by lack of sunlight.[10]

Unfortunately, however, concomitant calcium, 25(OH)vitamin D, and $1,25(OH)_2$vitamin D levels could not be determined during this study. As a result, the major question regarding vitamin D remains unresolved, that of the exact role played by vitamin D in observed calcium metabolism during submarine patrols. The problem lies in the fact that studies of the individual metabolites along the pathway, vitamin D in the case of this study and 25(OH)vitamin D in the case of the Preece et al studies, cannot give a complete representation of calcium/vitamin D economy. For example, Davies' proposal assumes that 25(OH)vitamin D levels directly affect calcium levels. On the contrary, it was indicated earlier in this presentation that 25(OH)vitamin D has practically no biological activity and that $1,25(OH)_2$vitamin D is the biologically active compound.

A non-submarine study by Adams in 1982 is the most complete assessment available which describes the interrelationships between the various $1,25(OH)_2$vitamin D precursors and illustrates the necessity for more comprehensive investigations in the submarine environment. In the study he compared vitamin D, 25(OH)vitamin D, $1,25(OH)_2$vitamin D, and parathyroid hormone levels in normal controls and in vitamin D deficient subjects before and after exposure to various amounts of ultraviolet light. Calcium levels were not measured. The results are summarized in Table III below:

TABLE III

Control vs. vitamin D deficient subjects
after UV light exposure

	vitamin D	25(OH)D	$1,25(OH)_2D$	PTH
Controls	dose	slight	no change	
Subjects	dependent	slight		
Baseline (reference 1)	contr>subject	contr>>>subj	contr subj	

In light of these findings, he concluded that 25(OH)vitamin D levels were most reflective of overall vitamin D status since baseline levels differed so markedly between the groups. Vitamin D levels, on the other hand, represented a dose-dependent reflection of recent ultraviolet light exposure and interval between exposures. $1,25(OH)_2$vitamin D levels represented the response to calcium and phosphate levels via parathyroid hormone. These findings and conclusions were consistent with results of other studies in which 25(OH)vitamin D levels were shown to have a seasonal variance, with the maximum in September and minimum in mid-winter, and also to vary with diet and degree of sunlight exposure[23,25,43] or which showed that $1,25(OH)_2$vitamin D levels had no diet, sunlight, or seasonal dependence.[25] In summary, current thought regarding $1,25(OH)_2$-vitamin D and its precursors is that (1) vitamin D levels reflect recent substrate availability (thus

making it the logical choice for the absorption study presented earlier), (2) 25(OH)vitamin D levels reflect overall vitamin D status over time, and (3) $1,25(OH)_2$vitamin D levels reflect the instantaneous hormonal response to calcium/phosphate status.

Despite the above conclusions, however, there appears to be evidence from several sources for a more immediate, active role of sunlight in calcium homeostasis. Neer, in 1971, exposed elderly males in a nursing home without significant sunlight exposure to controlled amounts of full spectrum lighting. He found that calcium absorption increased by 15% in those exposed and decreased by 25% in unexposed controls and concluded that the diet in this group(elderly males in nursing homes) is often inadequate to compensate for lack of sunlight.[32] Vitamin D levels were not measured. Davies' chamber study presented previously indicated an increase in fecal calcium loss which paralleled the decreasing 25(OH)vitamin D levels.[11] Adams' study itself showed that in vitamin D deficient individuals exposed to ultraviolet light there was a remarkable increase in levels of $1,25(OH)_2$vitamin D, the compound which directly regulates calcium levels, with only a mild change in 25(OH)vitamin D levels. Apparently ultraviolet light has a more immediate effect on calcium metabolism in certain situations, most notably in conditions of decreased 25(OH)vitamin D levels. These findings support the premise that there is a certain low level of 25(OH)vitamin D, caused by lack of sunlight or inadequate diet or both, below which calcium metabolism is affected regardless of parathyroid hormone input - a condition which, if chronic, causes rickets in children and osteomalacia in adults.[32]

It is highly unlikely that the young, healthy population of submariners is at any significant risk for osteomalacia; there have been no reported cases of the disease in submariners. Yet, if 25(OH)vitamin D levels decrease over one patrol to a point where calcium metabolism is affected, one wonders about the effect of repeated patrols over a 20-30 year career. Obviously, in order to clarify the role of vitamin D in the calcium behavior noted on extended submarine patrols, a single study simultaneously investigating vitamin D, 25(OH)vitamin D, $1,25(OH)_2$vitamin D, parathyroid hormone, serum calcium, and urine calcium excretion appears indicated. In addition, such a study, if done on a U.S. submarine, would allow a comparison to Preece's British submarine study regarding the effects of the vitamin D fortified American diet versus the unfortified British diet on 25(OH)vitamin D levels. If a significant derangement with potentially clinical implications is identified, perhaps vitamin D supplementation may be indicated. It has already been shown in this paper that absorption of such supplements would not be impaired on patrol.

Another area that merits further study is the calcium/CO_2 relationship. Recent advances in atmosphere control equipment on board submarines have resulted in progressively lower ambient CO_2 levels. As an example, the CO_2 levels on the patrol used for this study showed an average daily maximum of 3.3 torr and an average daily minimum of 1.7 torr, an effective CO_2 percentage of 0.22 - 0.43%, significantly lower than the levels found in the early calcium studies of the 1960's and 1970's. It would be logical to

expect that the effect on calcium levels and excretion would be smaller than the previous studies. If such a result were shown to occur, then a calcium/vitamin D study performed during the same or a similar patrol would have a lower possibility of interference from the CO_2 influence known to occur in early and suspected in late patrol. The relatively sudden decrease in calcium and acid excretion which takes place at approximately 7 weeks and continues into recovery is intriguing and as yet unresolved. It appears that, in order to resolve the issue, a combined CO_2/vitamin D calcium investigation measuring all appropriate parameters during patrol and into the post-patrol period, in which some of the subjects continued seclusion from sunlight, would be necessary. Such a logistically complex undertaking would very likely prove to be impractical on an operational submarine and crew, but, in view of the potential information to be gained, it is worth consideration.

Perhaps the most significant area for discussion and speculation provoked by this study and the calcium/vitamin D relationship in general is full spectrum lighting itself. The significance lies not merely in the potential for gathering data of academic interest but in the possibility for some very practical applications to the submarine force. Based on the evidence from this study and the 25(OH)vitamin D studies of Preece et al, one can conclude that it is the lack of sunlight exposure on extended patrols that causes the substantial drop in 25(OH)-vitamin D levels. The actual clinical significance of this drop has yet to be deter- mined. However, if such a significant (statistically) decrease occurs, then there is the strong possibility that other sunlight related biochemical or physical parameters are affected. It is well known that sunlight induces many direct and indirect biochemical or physiologic responses in the body, such as sunburn, photosensitivity reactions, melanin, and vitamin D synthesis[48], and melatonin production by the pineal gland. Less obvious and explainable are findings by several investigators of increased 30 yard dash performance[5] or isometric exercise performance[42] after single exposures to ultraviolet light. Using full spectrum lighting, artificial lighting designed to simulate the natural spectrum of sunlight, other workers have also shown a positive effect on strength, athletic performance[28], decreasing fatigue[29], academic performance[6], and vertical jumping ability compared to artifical light. Although the mechanism for these phenomena is not clear, it is apparent that there is some component of the full spectrum of natural light, not contained in conventional artificial incandescent or fluorescent lighting, which has a positive influence on performance.

Based on the above findings, home, office, and industrial designers have developed an increased awareness of the importance of full spectrum lighting. Formerly their emphasis was in meeting purely visual needs - optimum illumination, glare reduction, efficiency. Now the emphasis appears to be in addressing the overall physiological needs of the individual with more windows, skylights, etc.[48] The exact nature of these physiological needs, however, is not clearly delineated. Nevertheless, much effort has been expended in the identification of potential hazards from the various chemical and physical constituents of our environment, including those on submarines, and comparatively little has been expended in addressing sun-

light itself, such a vital part of that environment. We are only beginning to explore the effects of the biologically unnatural lighting environment, an environment to which many individual in the developed countries are increasingly exposed an in which certain groups may be particularly at risk, such as the elderly, the chronically ill, or even some submariners. Investigation could not only assess the potential harmful effects of lack of natural light (e.g. bone demineralization) but also study the less tangible but possibly beneficial effects of natural light versus artifical light (e.g. performance).

This particular area of research has ramifications specific to the submarine. First, the submarine, by the very nature of its mission, offers an ideal testing platform for full spectrum lighting studies over the expense, inconvenience, and potential loss of control of surface studies. In light of the recent interest in such studies, the submarine, within operational constraints of course, can potentially fill the need for healthy subjects in a controlled environment. Second, and more important, is the attention given to the effects of full spectrum lighting on performance. The supportive evidence is by no means conclusive. However, if such positive influence on performance can be supported further in submarine studies, the potential immediate benefit to the submarine force could be significant. In such a strategic arm of the military, in which optimum functioning of personnel and equipment is not only a major goal but quite possibly vital to survival, such research may help provide or enhance an advantage in personnel effectiveness.

CONCLUSION

Utilizing historical perspective, an actual research project, and a degree of speculation, this paper has been an attempt to discuss the calcium and vitamin D metabolism on submarine patrols - presenting findings of the present and previous studies, evaluating the theories and proposals, and discussing their implications and potential benefits.

The early finding of a significant decrease in renal calcium excretion led to rather unconventional theories related to acid-base homeostasis. A link was established between the elevated levels of ambient CO_2 and the calcium findings and could not be explained on the basis of renal compensation alone. As a result of further research, a bone-buffering system involving both calcium and CO_2 and operating at an acid (CO_2) load lower than the threshold required for renal compensation was implicated. This cyclic, slow-turnover deposition and release of calcium, linked to CO_2, in response to chronic low level hypercapnia readily explained the calcium behavior. The continued suppression of calcium excretion into the recovery period remained a puzzle and alternative explanations were sought.

Recent successes in tracing the vitamin D metabolic pathways and establishing reliable assay methods made possible the investigation of the contributory effect of vitamin D. Findings by Preece et al suggest that chronic sunlight deprivation over 60-70 days causes a significant decrease in 25(OH)vitamin D stores in the body and may explain the calcium excretion findings in the late patrol and post patrol periods. The vitamin D absorp-

tion study presented in this paper further supports the cause of the 25(OH)vitamin D decrease as sunlight deprivation. Both the bone-buffering mechanism and the vitamin D mechanism offer workable explanations and, in fact, supplement one another. It is likely that both are contributory. Further work, perhaps involving $1,25(OH)_2$vitamin D and full spectrum lighting, is required to characterize more fully the calcium/vitamin D relationship and its influence on the overall calcium metabolism on submarine patrols.

Although it is a relatively established fact that the overall health of nuclear submariners is not affected by the submarine environment, such research, supplemented by mineralization studies, may identify specific at-risk groups for which some kind of supplementation would be indicated. Even if no ill effects are discovered in the young, healthy submarine population, such work could definitiely benefit other already identified at-risk subgroups, such as elderly males and post-menopausal females, in terms of sunlight effects on vitamin D economy. Finally, the additional investigations may indeed indicate possibilities for the enhancement of performance and productivity of workers in the sunlight-free environment, with direct potential advantage to the submarine force.

This paper has shown that the initial concern for altered calcium homeostasis noted in early nuclear submarines has led to several different, and possibly far-reaching, lines of investigation. Submarine studies have contributed significantly to the understanding of calcium and vitamin D interrelationships. It is hoped that the vitamin D absorption study presented in this paper has offered a worthwhile and useful addition to the overall research effort. In light of their potential usefulness, further investigations appear warranted and the submarine environment offers the unique opportunity to advance knowledge and applications in this area.

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A 42% decrease in 25(OH)vitamin D levels has been noted in subjects over the course of submarine patrols and is thought to be the result of prolonged sunlight deprivation. The influence of the submarine environment on absorption of ingested vitamin D has not been previously investigated. This study examines the vitamin D absorption patterns of 5 subjects at the beginning, middle, and end of a 69-day patrol on a normal diet and with no exposure to sunlight. It was found that the magnitude and pattern of

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absorption does not change appreciably. It is concluded that the absorption of vitamin D is not affected by the submarine environment and that any drop in 25(OH) vitamin D levels seen during patrol is caused by lack of sunlight, ultraviolet light in particular. Implications in terms of sunlight deprivation and calcium metabolism are discussed in a historical review of calcium and vitamin D investigations on submarines.